[00099] Regional blood flow was calculated from optical absorbance (AU) measurements corrected by tissue weight as follows:

$$\text{Flow to sample (mL/min/g)} = \frac{(\text{AU/sample})(\text{reference withdrawal rate})/\text{wt.}}{(\text{AU/reference sample})}$$

[000100] To evaluate further the angiogenic potential of intrapericardial FGF-2 in chronic myocardial ischemia, regional myocardial blood flow was measured at different time points using colored microspheres. Three weeks after implantation of ameroid occluders, at the time of intrapericardial drug delivery, resting myocardial blood flow in the LCX territory was similar in all treatment groups [baseline coronary flow (ml/min/g): 1.00 ± 0.31 in controls and 0.97 ± 0.23 in heparin-treated animals versus 0.92 ± 0.08 in the 30 μg FGF-2 group, 0.99 ± 0.15 in the 200 μg FGF-2 group, and 1.10 ± 0.14 in the 2 mg FGF-2 group, $P = .94$] and was significantly lower than flow in the LAD territory (LCX flow: 1.00 ± 0.35 ml/min/g versus LAD flow: 1.43 ± 0.43 ml/min/g, $P < .0001$). Four weeks after intrapericardial drug delivery, LCX flow was significantly higher in FGF-2-treated animals than in controls and heparin-treated animals (ANOVA $P = .002$). At the time of the final study, coronary flow (ml/min/g) was 1.05 ± 0.21 in controls ($P = .7$ compared with baseline) and 1.09 ± 0.13 in the heparin group ($P = .19$ compared with baseline and $P = .6$ compared with controls) versus 1.31 ± 0.12 in the 30 μg FGF-2 group ($P = .0001$ compared with baseline and $P = .004$ compared with controls), 1.25 ± 0.15 in the 200 μg FGF-2 group ($P = .002$ compared with baseline and $P = .03$ compared with controls), and 1.32 ± 0.16 in the 2 mg FGF-2 group ($P = .004$ compared with baseline and $P = .005$ compared with controls).

[000101] **MRI.** MRI was performed on all animals at the time of treatment initiation and at the time of final study. MRI was carried out in the body coil of a 1.5 Tesla whole body Siemens Vision system (Iselin, NJ) as previously described. The following measurements were performed:

a. Determination of resting left ventricular EF (%).

NAME alone (60 ± 9 and 55 ± 8%, respectively) and hearts perfused with L-NIL alone (57 ± 9 and 67 ± 4%, respectively).

[000112] Unlike initial pretreatment with rFGF-2, addition of the growth factor to the coronary perfusate after the onset of ischemia, immediately before reperfusion, did not improve LV function 20 min after reperfusion (LVP 60 ± 4%, dP/dt_{max} 62 ± 4%, and dP/dt_{min} 58 ± 4%, all P = NS vs. control). As in the case of acute ischemic changes, pretreatment with either L-NAME or L-NIL led to a complete inhibition of rFGF-2 effects (Figs. 2 and 3).

[000113] *Isolated Heart Preparation* Hearts were excised from adult C57/BL6 mice of either sex that had been anesthetized and heparinized (500 U/100 g body wt). The aorta was slipped over a 20-gauge blunt-tipped stainless steel needle through which oxygenated (95% O_2-5% CO_2) Krebs-Henseleit (KH) buffer (in mM: 118.0 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.5 $CaCl_2$, 1.2 $MgCl_2$, 23.0 $NaHCO_3$, 10.0 dextrose, and 0.3 EDTA, pH 7.4) was pumped at a rate of ~3 ml/min. An intraventricular balloon catheter system specially designed for the mouse heart was passed through the mitral annulus into the left ventricle, and the distal end of the balloon catheter was connected to a Statham P23b (Gould, Cleveland, OH) transducer to record intraventricular pressure. Left ventricular (LV) pressure recordings were analyzed with regard to LV developed pressure (LVP), LV end-diastolic pressure, peak rate of pressure development (dP/dt_{max}), time to 90% pressure decline, and peak rate of pressure decline (dP/dt_{min}).

[000114] *Ischemia and reperfusion.* The hearts were subjected to no-flow ischemia for 15 min. The organ bath was evacuated of its oxygenated solution and refilled with nitrogen-saturated perfusate. Pacing was maintained during ischemia. LV pressure was monitored throughout ischemia and reperfusion. All hearts ceased to contract within 3 min. The time for LVP to fall to 10% of baseline (T_{LVP10}) was measured to quantify differences in LV function during early ischemia. Mean ischemic Ca_i^{2+} was calculated as the mean Ca_i^{2+} recorded from the 2nd through the 14th minute of ischemia. Contracture was defined as an

[000125] *Baseline Conditions and Effects of Ischemia* Baseline parameters of cardiac function including myocardial Ca_i^{2+} were similar at baseline in all groups and were not affected by administration of L-NAME, L-NIL (not shown), or rFGF-2. Interruption of coronary flow led to an abrupt fall in LV pressure in all hearts. This fall in LV pressure during early ischemia was significantly attenuated in hearts pretreated with rFGF-2 compared with control hearts. Pretreatment with rFGF-2 prolonged T_{LVP10} (124 ± 9 vs. 74 ± 5 s, rFGF-2 vs. control, $P < 0.05$) and significantly delayed the onset of contracture (893 ± 7 vs. 819 ± 36 s, rFGF-2 vs. control, $P < 0.01$).

[000126] To explore the role of NO in mediation of this cardioprotective effect of FGF-2, L-NAME was used to inhibit all isoforms of NOS in the heart. Pretreatment with L-NAME completely blocked the cardioprotective effects of rFGF-2 during ischemia, significantly reducing T_{LVP10} (79 ± 2 vs. 124 ± 9 s, L-NAME + rFGF-2 vs. rFGF-2, $P < 0.05$) and accelerating the onset of ischemic contracture (674 ± 24 vs. 893 ± 7 s, L-NAME + rFGF-2 vs. rFGF-2, $P < 0.05$). However, perfusion with L-NAME alone (in the absence of rFGF-2) did not affect either T_{LVP10} [69 ± 3 vs. 74 ± 5 s, L-NAME vs. control, P = not significant (NS)] or the onset of ischemic contracture (820 ± 24 vs. 819 ± 36 s, L-NAME vs. control, P = NS).

[000127] To further define the type of NOS enzyme involved in this FGF-2 response, a NOS2-selective inhibitor, L-NIL, was used. Similarly to L-NAME, L-NIL fully inhibited the cardioprotective effects of rFGF-2, significantly reducing T_{LVP10} (62 ± 3 vs. 124 ± 9 s, L-NIL + rFGF-2 vs. rFGF-2, $P < 0.05$) and accelerating the onset of ischemic contracture (652 ± 16 vs. 893 ± 7 s, L-NIL + rFGF-2 vs. rFGF-2, $P < 0.05$). Similarly to perfusion with L-NAME, perfusion with L-NIL alone, in the absence of rFGF-2, did not affect either T_{LVP10} (67 ± 6 vs. 74 ± 5 s, L-NIL vs. control, P = NS) or the onset of ischemic contracture (740 ± 39 vs. 819 ± 36 s, L-NIL vs. control, P = NS).

Example 4 Efficacy of Intracoronary Versus Intravenous FGF-2 an a Porcine Model Of Chronic Myocardial Ischemia

MRI, and had functional significance because it was accompanied by an increase in EF and improvement in target wall motion and target wall thickening in the high-dose intracoronary group. The effect on EF was added to the natural tendency to grow collaterals and improve perfusion and function of ischemic myocardium.

[000145] The current study presents evidence that a single intracoronary injection of 120 to 150 μg FGF-2 improves regional blood flow as well as regional and global cardiac function. The ineffectiveness of intravenous FGF-2 might result from less favorable pharmacokinetics. Several studies have reported a 3- to 10-fold lower recovery of radiolabeled FGF-2 from the myocardium after intravenous administration than after intracoronary injection, which in turn has a lower recovery and shorter redistribution times than intrapericardially delivered FGF-2. FGF-2 might be retained in the myocardium by a high-capacity, low-affinity sink provided by heparin sulfates in the matrix and on the surface of endothelial cells, which are upregulated by ischemia. In addition, expression of FGF-R1 receptors, which are the primary transducers of FGF-2 signaling, is also upregulated by ischemia.

[000146] In this animal study, in accordance with the phase I clinical trial, intravenous FGF-2 and 2 μg/kg intracoronary FGF-2 had no major hemodynamic, hematologic, or biochemical side effects.

[000147] Clinical implications If a single intracoronary infusion of FGF-2 proves to be effective in patients with chronically ischemic myocardium, this strategy will greatly increase the number of patients that might benefit from adjunctive growth factor therapy, especially in view of the minimal side effects. Each patient undergoing percutaneous revascularization is a candidate for angiogenic therapy because most interventions are local and aimed at the most severe stenoses in epicardial arteries. The additional benefit of myocardial salvage during reperfusion injury by FGF-2 further emphasizes the potential value of this adjunct pharmacotherapy.

[000165] Because of the protracted course of new collateral development, the potential for hemodynamic disturbances associated with bolus intravascular delivery, and the possibility for toxicity from elevated circulating levels of angiogenic growth factors, a local sustained bFGF delivery strategy using heparin-alginate microcapsules was used. This delivery system allows prolonged (4 to 6 weeks) sustained release (first-order kinetics). In animal studies, there was a dose-dependent effect of bFGF that was not associated with detectable serum levels, hemodynamic effects, or local or systemic toxicity.

[000166] Of the 46 patients judged to have a major coronary artery that could not be grafted on the basis of angiographic appearance, 22 patients were actually successfully grafted at the time of CABG. Thus, preoperative assessment of arterial suitability for bypass proved to be inaccurate in almost 50% of cases. In accordance with prior observations, the major epicardial artery most likely to be unsuitable for grafting was the RCA. In no case was the LAD considered ungraftable. This paucity of LAD cases is probably a reflection of the reluctance to refer those patients in whom the LAD may not be bypassed for surgical intervention.

[000167] The combination CABG/bFGF therapy was not associated with an excess rate of complications. Two operative deaths in this study most likely reflect the higher operative risk in patients with advanced coronary disease and left ventricular dysfunction who have incomplete revascularization. The absence of hemodynamic abnormalities associated with heparin-alginate bFGF delivery is consistent with the undetectable serum levels of bFGF at any time after growth factor administration. In addition, the lack of short- or intermediate-term adverse effects on serum chemistries, hematologic profile, liver function tests, or urinalysis also suggests that this mode of delivery is not associated with systemic toxicity. These observations therefore emphasize the safety of heparin-alginate bFGF delivery at the time of CABG.

[000168] this randomized, double-blind, placebo-controlled study of bFGF in patients undergoing CABG demonstrates the safety and feasibility of this mode